Neuron
Previews

Cadherins as Matchmakers

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DOI 10.1016/j.neuron.2011.08.005

Cadherins implement afferent-target matching in invertebrates, but proof for this concept in mammalian circuits has remained elusive. Two new studies in this issue of *Neuron* show that cadherin-6 mediates retinal ganglion cell target selection and that cadherin-9 promotes synapse specificity in the hippocampus.

To establish functionally precise patterns of connectivity, afferent axons must target select regions and, at the end of the line, synapse with specific cellular partners. The cadherins are calcium-dependent cell adhesion molecules. Among the many cadherin types—classical (type I and II) cadherins, desmosomal cadherins, atypical cadherins, and protocadherins—N-cadherin (a classical type I cadherin) has been the most studied and has been implicated in cellular processes ranging from tissue coherence and boundary formation to axon guidance, fasciculation, and laminar targeting (Redies, 2000; Takeichi, 2007). In addition, cadherins are localized at both pre- and postsynaptic terminals, making them ideal participants in synapse formation (Arikkath and Reichardt, 2008; Sanes and Yamagata, 2009). Experiments on the chick and fly visual systems have argued for cadherin-mediated organization of axons for proper targeting and innervation of specific lamina (Clandinin and Feldheim, 2009), but convincing evidence for axon-target matching through cadherins in mammalian systems is lacking.

Over the last few years, the cadherin hypothesis of target selection in mammalian neurons has lost momentum. First, the approaches used in invertebrates and lower vertebrates are difficult to apply to the mammalian nervous system: conventional knockouts are usually early embryonic lethal or have no apparent phenotype, and dominant-negative approaches often produce inconclusive or nonspecific effects (Redies, 2000; Takeichi, 2007). Recently, because of their potential for diversity of multiple isoforms similar to Dscams in invertebrates, the protocadherins have entered the limelight as candidates for chemoaffinity (Zipursky and Sanes, 2010), but to date these molecules have not lived up to their promise.

In this issue of *Neuron*, cadherins make a comeback as mediators of mammalian axon-target recognition. The study by Osterhout et al. (2011) investigates the mechanisms of cell-cell matching in the mammalian visual system, focusing specifically on the role of cadherins in the innervation of select visual nuclei by a subset of non-image-forming retinal ganglion cells (RGCs) (Figure 1A). Although many molecules have been identified for guidance to and topographic organization within targets (Atkinson-Leadbeater and McFarlane, 2011; Clandinin and Feldheim, 2009), there is scant information on how retinal axons choose among several possible targets in the visual thalamus and midbrain. Recently, Su et al. (2011) reported targeting defects of non-image-forming RGCs to the ventral lateral geniculate nucleus and intergeniculate leaflet in knockouts of the extracellular matrix molecule Reelin, but the underlying molecular mechanism for Reelin-mediated matching is not clear.

Osterhout et al. report that cadherin-6 (Cdh6) directs a subset of RGCs to connect with specific retinorecipient target nuclei, potentially through cadherin-cadherin matching. Analysis of the expression pattern of classical cadherins (cadherin-1 through 8) in the visual pathway revealed that Cdh6 is specifically expressed in non-image-forming retinorecipient nuclei during RGC target innervation (E18 to P4) (Figure 1A). To trace axons, the authors used a combination of cadherin-6 loss-of-function mice and transgenic mouse lines with genetically labeled subsets of RGCs. A line of BAC-GFP-transgenic mice revealed that cadherin3 (Cdh3)-expressing RGCs selectively innervate targets expressing Cdh6, even though Cdh3 is not expressed in these targets (Figure 1A). All Cdh3+ RGCs express Cdh6, but some Cdh6+ RGCs do not express Cdh3 and these latter RGCs project to additional targets (Figure 1A). By crossing Cdh6 knockout (KO) mice with the Cdh3:BAC GFP mice, Osterhout et al. were able to show defects in the targeting specificity of Cdh3+ RGCs. In Cdh6 KO mice, Cdh3+ RGCs fail to recognize and halt at their targets such as the optic pretectal nucleus (OPN) and overshoot to the superior colliculus (SC). The authors argue that the targeting errors reflect defects in Cdh6 homophilic recognition between RGC axons and target neurons rather than perturbations in Cdh6-mediated target nuclei formation, as the organization of the OPN seems normal.

The Osterhout et al. report provides strong evidence for linking types of RGCs to their specific targets based on cadherin-6 expression and is the first report in mouse of central targeting defects associated with classical cadherin function. Nonetheless, the precise role of Cdh6 has yet to be sorted out. Does it act through axon-target recognition, as suggested by the authors, or through axon-axon interactions during extension, as proposed for the atypical cadherin Flamingo, where differences in levels of homophilic adhesion between growth cones and axons influence their trajectory

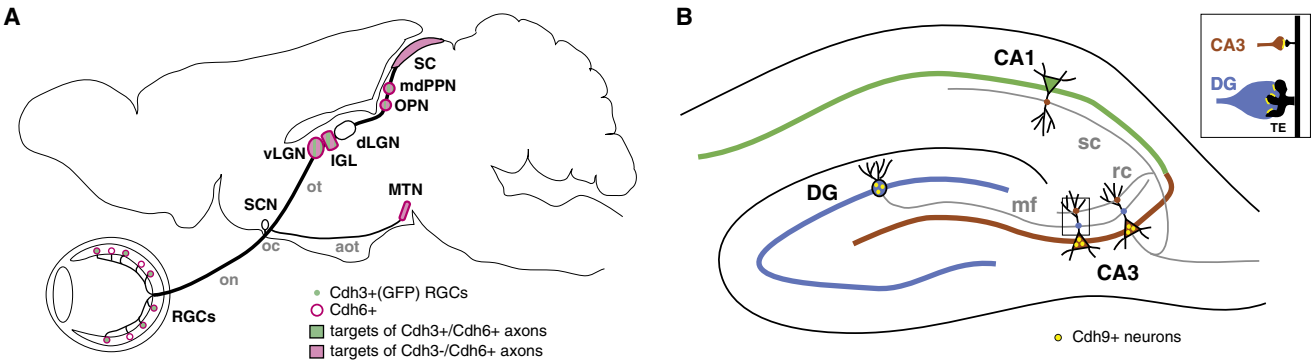

Figure 1. Cadherin Expression in the Mouse Visual Pathway and Hippocampus

(A) In the mammalian visual system, retinal ganglion cell (RGC) axons exit the retina and form the optic nerve (on), cross or avoid the optic chiasm (oc), regroup in the optic tract (ot), and then innervate different target regions. The main RGC targets are the dorsal lateral geniculate nuclei (dLGN) and the superior colliculus (SC), but none receive inputs from Cdh3+ axons. RGC axons also project to various other nuclei important for non-image-forming but visually mediated functions such as the suprachiasmatic nucleus (SCN), the ventral lateral geniculate nucleus (vLGN), the intergeniculate leaflet (IGL), the olivary pretectal nuclei (OPN), the medial division of the posterior pretectal nucleus (mdPPN), and the medial terminal nucleus (MTN) via the accessory optic tract (aot). In the retina, Cdh6 is expressed in a subset of non-image-forming RGCs, many of which coexpress Cdh3. Cdh6, but not Cdh3, is expressed in most of the targets of Cdh3+ RGCs. The visual targets receiving Cdh3+/Cdh6+ and/or Cdh3−/Cdh6+ RGC axons are shown.

(B) Mossy fibers (mf) extend from granule cells in the dentate gyrus (DG) and innervate pyramidal neurons in CA3. Mossy axons develop large, lobulated presynaptic terminals that form asymmetric synapses onto CA3 dendrites near the soma, on multiheaded spines called thorny excrescences (TE). CA3 neurons also project recurrent collaterals (rc) on CA3 distal dendrites and Schaffer collaterals (sc) that project onto CA1 neurons. DG and CA3 neurons express Cdh9.

to specific targets in the fly eye (Chen and Clandinin, 2008)? Such a mechanism might explain the defects in target overshooting observed in the Cdh6 KO. Is Cdh6 expression important in RGCs, target cells, or both, for targeting toward the OPN? Would expression of Cdh6 in other RGCs be sufficient to change targeting toward the Cdh6-expressing nuclei? Although the Cdh3-GFP mouse is a good tool for tracing the projection defect, the fact that cadherin-3 and cadherin-6 are coexpressed in the same RGCs raises the possibility that combinatorial interactions of different cadherins could function in matching axon to target (Shimoyama et al., 2000; Shapiro et al., 2007) and could explain why the loss-of-function phenotype is not fully penetrant. It will be interesting to determine whether similar targeting defects exist in cadherin-3 mutants and to characterize other cadherin-expressing RGC subpopulations, to divine whether there is a "cadherin code" for targeting by different subtypes of RGCs.

Some of these questions are answered in the study by Williams et al. (2011), but in a different system and at the level of the synapse. Williams et al. used the well-characterized hippocampal neural circuitry as a model of synapse formation to investigate mechanisms underlying the preference of dentate gyrus (DG) axons to synapse onto CA3 pyramidal neurons (Figure 1B). Although previous work hinted at a role for cadherins in the establishment of the mossy fiber pathway (Bekirov et al., 2002, 2008), the data in Williams et al. comprise the first direct evidence that cadherins regulate the formation of synapse between DG neurons and CA3 neurons.

By using a clever in vitro assay, where dissociated hippocampal cells (DG, CA1, and CA3) are plated as "microislands" and identified with specific markers (Prox1, CTIP2, PY), the authors were able to observe and manipulate interactions between a small number of neurons. In microislands where only one hippocampal DG neuron is transfected by synaptophysin-GFP, Williams et al. addressed the selectivity of innervation of hippocampal cell types by DG neurons. Even though DG axons do not grow preferentially to CA3 axons and contact dendrites of other DG and CA1 cells, they make synapses preferentially onto their correct CA3 targets in this culture setting. Furthermore, paired electrophysiological recordings confirm the functional synaptic bias of DG axons for CA3 neurons. Thus, the authors handily demonstrate that this assay is able to recapitulate the preferential synaptic innervation of CA3 neurons by DG axons, a boon for future studies of hippocampal circuitry.

To determine whether DG-CA3 synapse specificity is due to increased synaptogenic tendencies or reduced elimination of DG-CA3 synapses, the authors used a "synaptoporin assay." DG neuron synapses express both VGLUT1 and synaptoporin, whereas CA1 and CA3 neurons express only VGLUT1. By coimmunostaining for synaptoporin and VGLUT1, the authors were able to examine synaptic development between hippocampal cell types (as identified by cell-specific markers) in vitro. At each time point examined, CA3 neurons formed significantly more synapses with DG neurons than with CA1 neurons, though CA1 and CA3 neurons formed equivalent numbers of synapses in total. In addition, DG-CA3 synapses were much larger than regular excitatory synapses, as in vivo. Thus, the authors could argue with conviction for selective synapse formation onto correct targets, and not elimination of incorrect synapses.

Williams et al. postulated that such specific synapse formation must be mediated by a transmembrane protein with an extracellular domain that could participate in cell-cell interactions. Based on the analysis of gene-expression profiles, the authors identified cadherin-9 (Cdh9), which is highly expressed in both DG and CA3 neurons, as an ideal candidate for such synaptogenic specificity. Cdh9 protein is found in puncta adjacent to

active zones, is capable of homophilic interaction in a calcium-dependent manner, and can recruit β-catenin. The next important result was that transfection of Cdh9 shRNA in postsynaptic neurons in vitro leads to a reduction in DG synapses on CA3 neurons, but not on CA1 neurons. However, overexpression of Cdh9 in the various cell types did not cause an increase in DG synapses, implying that Cdh9 is not sufficient to drive synapse formation per se as previously determined for other cadherins (Arikkath and Reichardt, 2008). These results suggest that the expression of cadherin-9 in CA3 neurons is crucial for the preferential synaptic innervation by DG axons, and indeed, loss of cadherin-9 in DG neurons in vivo by lentivirus or in utero electroporation during development caused decreased mossy fiber bouton size and perturbed morphology, including a reduction in presynaptic filopodia.

The definitive experiment in substantiating cadherin-9's potential function in synapse specificity was to knock down Cdh9 only in CA3 neurons in vivo, which resulted in CA3 dendrites with long, filopodia-like processes instead of the squat, lobular thorny excrescences contacted by DG mossy fiber endings. Non-cell-autonomous effects were also observed, with much smaller mossy fiber boutons developing on DG neurons, similar to when cadherin-9 is knocked down in DG neurons. These data strongly implicate cadherin-9 in sculpting synaptic structure and preventing new protrusions and/or synapses from forming. The authors propose that as a result of having a defective adhesion system for synaptic targeting, filopodia continue to search for partners and therefore extend after Cdh9 knockdown in CA3 neurons because they lack the ability to form and maintain synaptic contacts. These in vivo results show that cadherin-9 acts at both pre- and postsynaptic sites for specific DG-CA3 synapse formation, through its homophilic interactions and cell-autonomous and non-cell-autonomous effects.

As with any study on a previously known molecule investigated with new tools, further questions arise. The authors argue for selective synapse formation rather than an elimination of inappropriate inputs, but how the preferential innervation occurs in vivo still needs to be determined. Moreover, it is still not clear how cadherin-9 acts on pre- and postsynaptic cells to achieve the remarkable morphogenesis of stereotypic presynaptic mossy boutons and the postsynaptic thorny excrescences on which they terminate.

Nonetheless, Osterhout et al. and Williams et al. present two elegant examples of cadherin-mediated axon-target matching. Classical cadherins are thought to mediate adhesion through their homophilic interactions. However, type II cadherins can engage in heterophilic interactions (Shimoyama et al., 2000), and given that multiple cadherins are expressed in both the visual system and hippocampus, heterophilic interactions could also be at play in axon-target matching. Future analysis of the structural organization of cadherins and interactions of their different domains will hopefully extend our knowledge of such heterophilic cadherin-mediated target-matching mechanisms.

The critical next question for both studies is to identify the downstream effectors of cadherins important for these phenomena. Catenins are likely candidates, but more precise experiments are now needed to determine how the matchmaker succeeds in ensuring a happy union.

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